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ISO 2962 - 1984
(Reaffirmed 1999)

Indian Standard

CHEESE AND CHEESE PRODUCTS— DETERMINATION OF TOTAL PHOSPHORUS CONTENT—MOLECULAR ABSORPTION SPECTROMETRIC METHOD

UDC 637·3 : 543·42 : 546·18

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NEW DELHI 110002

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IS 12756 : 1989
ISO 2962 - 1984

Indian Standard

CHEESE AND CHEESE PRODUCTS — DETERMINATION OF TOTAL PHOSPHORUS CONTENT — MOLECULAR ABSORPTION SPECTROMETRIC METHOD

NATIONAL FOREWORD

This Indian Standard, which is identical with ISO 2962-1984 'Cheese and processed cheese products — Determination of total phosphorus content — Molecular absorption spectrometric method', issued by the International Organization for Standardization (ISO), was adopted by the Bureau of Indian Standards on 31 August 1989 on the recommendation of the Dairy Products Sectional Committee (AFDC 34) and approval of the Food and Agriculture Division Council.

In the adopted standard, certain terminology and conventions are not identical with those used in Indian Standards; attention is particularly drawn to the following:

a) Wherever the words 'International Standard' appear, referring to this standard, they should be read as 'Indian Standard'; and

b) Comma (,) has been used as decimal marker while in Indian Standards, the current practice is to use point (.) as the decimal marker.

CROSS REFERENCE

International Standard	*Corresponding Indian Standard*
ISO 707-1985 Milk and milk products — Sampling	IS : 11546-1985 Methods of sampling of milk and milk products (Identical)

In reporting the result of a test or analysis made in accordance with the standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960 'Rules for rounding off numerical values (*revised*)'.

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1 Scope and field of application

This International Standard specifies a molecular absorption spectrometric method for the determination of the total phosphorus content of cheese. The method is applicable to all kinds of cheese and to processed cheese products.

2 Reference

ISO 707, *Milk and milk products — Methods of sampling.*

3 Principle

Digestion of the cheese by sulfuric acid and hydrogen peroxide.

Formation of molybdenum blue by addition of molybdateascorbic acid solution. Molecular absorption spectrometric measurement, at a wavelength of 820 nm, of the blue colour formed.

NOTE — Dry ashing may be used, provided that the procedure gives the same results as the wet digestion.

4 Reagents

All reagents shall be of recognized analytical grade. The water used shall be distilled or deionized water, free from phosphorus compounds.

4.1 Concentrated sulfuric acid (H_2SO_4), ρ_{20} = 1,84 g/ml.

4.2 Hydrogen peroxide, solution containing about 30 g of H_2O_2 per 100 ml.

4.3 Molybdate-ascorbic acid solution.

4.3.1 Sodium molybdate solution.

Dissolve 12,5 g of sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$) in 5 mol/l sulfuric acid solution, dilute with this sulfuric acid solution to 500 ml, and mix.

4.3.2 ascorbic acid, solution.

Dissolve 10 g of ascorbic acid ($C_6H_8O_6$) in water, dilute to 200 ml, and mix.

NOTE — This solution cannot be stored.

4.3.3 Mixed solution.

Immediately before use, mix 25 ml of solution 5.3.1 with 10 ml of solution 4.3.2, dilute to 100 ml with water, and mix.

4.4 Phosphorus, standard solution corresponding to 100 µg of P per ml.

Dry for at least 48 h about 1 g of potassium dihydrogenorthophosphate (KH_2PO_4) in a desiccator over an efficient desiccant, for example concentrated sulfuric acid.

Dissolve 0,439 4 g of the previously dried phosphate in water, dilute to 1 000 ml, and mix.

5 Apparatus

All glassware shall be thoroughly cleaned with a phosphorus-free detergent and rinsed with distilled water.

5.1 Analytical balance.

5.2 Device for grinding or grating cheese, capable of being easily cleaned.

5.3 Boiling water-bath.

5.4 Digestion flasks (Kjeldahl flasks or digestion tubes), of capacity 25 ml.

5.5 Heating apparatus.

5.5.1 Micro gas burners, for heating Kjeldahl flasks.

NOTE — Electric heaters may also be used.

5.5.2 Heating block, for heating digestion tubes.

5.6 Glass beads.

5.7 Graduated cylinders, of capacities 5 and 25 ml.

5.8 One-mark volumetric flasks, of capacities 50 and 100 ml.

5.9 One-mark pipettes, to deliver 1, 2, 3, 5 and 10 ml.

5.10 Spectrometer, suitable for measurements at a wavelength of 820 nm, equipped with cells of optical path length 10 mm.

6 Sampling

6.1 See ISO 707.

6.2 Store the sample in such a way that deterioration and change in composition are prevented.

7 Procedure

7.1 Preparation of the test sample

Remove the rind, smear or mouldy surface layer of cheese, in such a way as to provide a sample representative of the cheese as it is usually consumed. Grind or grate the sample by means of an appropriate device (5.2). Mix the ground or grated mass quickly, and if possible grind or grate a second time and again mix thoroughly. If the sample cannot be ground or grated, mix it thoroughly by intensive stirring and kneading.

Transfer the test sample to an airtight container to await analysis, which should be carried out as soon as possible after grinding or grating. If delay is unavoidable, take all precautions to ensure proper preservation of the sample and to prevent condensation of moisture on the inside surface of the container.

Clean the device after grinding or grating the sample.

7.2 Determination

7.2.1 Into a digestion flask (5.4) weigh, to the nearest 1 mg, a test portion of 0,5 to 1,0 g of the test sample.

Add three glass beads and 4 ml of the concentrated sulfuric acid (4.1).

NOTE — If the water content of the cheese is less than 50 % (*m/m*), a test portion of approximately 0,5 g is sufficient. In the case of fresh cheese, a test portion of approximately 1,0 g can be taken.

7.2.2 Heat the digestion flask under a well ventilated fume hood.

NOTE — A Kjedahl flask shall be placed in an inclined position.

Control the height of the flame so as to limit the production of foam in the flask. Foaming into the neck of the flask is allowed but the foam shall not escape.

Keep the mixture gently boiling. Avoid local overheating and heating the flask above the surface of the liquid contents.

7.2.3 As soon as the foaming stops, cool to room temperature. Carefully add a few drops of the hydrogen peroxide solution (4.2) and reheat.

Repeat this procedure until the contents have become clear and colourless. During heating, mix the contents from time to time by careful swirling. Avoid local overheating.

7.2.4 Rinse the neck of the flask with about 2 ml of water. Heat the contents again until the water has evaporated.

Allow the liquid to boil for 30 min in order to destroy traces of hydrogen peroxide. Avoid local overheating.

7.2.5 Cool to room temperature. Quantitatively transfer the liquid contents into a 100 ml one-mark volumetric flask (5.8). Dilute to the mark with water and mix well.

7.2.6 Pipette 1 ml of the solution into a 50 ml one-mark volumetric flask (5.8) and dilute with about 25 ml of water. Add 20 ml of the molybdate-ascorbic acid solution (4.3.3). Dilute to the mark with water and mix well.

7.2.7 Heat the flask in the water-bath (5.3) for 15 min.

7.2.8 Cool to room temperature in a cold water-bath. Within 1 h, measure the absorbance of the solution against that of the blank test solution (see 7.4) at a wavelength of 820 nm.

7.3 Calibration curve

7.3.1 Pipette 10 ml of the standard phosphorus solution (4.4) into a 100 ml one-mark volumetric flask (5.8). Dilute to the mark with water and mix well.

7.3.2 Pipette into a series of five 50 ml one-mark volumetric flasks (5.8) 0, 1, 2, 3 and 5 ml, respectively, of the diluted standard solution (7.3.1), i.e. equivalent to 0, 10, 20, 30 and 50 μg of P respectively. Dilute the contents of each flask to approximately 20 ml with water.

7.3.3 Add to the contents of each volumetric flask 20 ml of the molybdate-ascorbic acid solution (4.3.3). Dilute with water to the mark and mix well.

Proceed as specified in 7.2.7.

7.3.4 Cool to room temperature in cold water. Within 1 h, measure the absorbance of each of the calibration solutions against water as reference at a wavelength of 820 nm.

7.3.5 Plot these absorbances against the amounts of phosphorus added.

7.4 Blank test

Carry out a blank test by following the procedure specified in 7.2, but without a test portion.

8 Expression of results

8.1 Method of calculation

The total phosphorus content, expressed as a percentage by mass, is equal to

$$\frac{m_1}{100\,m_0}$$

where

m_0 is the mass, in grams, of the test portion;

m_1 is the mass, in micrograms, of phosphorus, read from the calibration curve (or calculated from the regression line obtained by the method of least squares).

Report the result to the second decimal place.

8.2 Repeatability

The difference between two single results obtained on identical test material by one analyst using the same apparatus within a short time interval should exceed 0,03 g of phosphorus per 100 g of product on average not more than once in 20 cases in the normal and correct operation of the method.

8.3 Reproducibility

The difference between two single and independent results obtained by two operators working in different laboratories on identical test material should exceed 0,06 g of phosphorus per 100 g of product on average not more than once in 20 cases in the normal and correct operation of the method.

9 Test report

The test report shall show the method used and the result obtained. It shall also mention all operating conditions not specified in this International Standard, or regarded as optional, as well as any incidents that may have influenced the result.

The test report shall include all the information necessary for the complete identification of the sample.

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